Mechanisms for Polycyclic Aromatic Hydrocarbon Degradation by Ligninolytic Fungi

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Ligninolytic fungi accomplish the partial degradation of numerous aromatic organopollutants. Their ability to degrade polycyclic aromatic hydrocarbons (PAHs) is particularly interesting because eukaryotes were previously considered to be unable to cleave fused-ring aromatics. Recent results indicate that extracellular peroxidases of these fungi are responsible for the intitial oxidation of PAHs. Fungal lignin peroxidases oxidize certain PAHs directly, whereas fungal manganese peroxidases co-oxidize them indirectly during enzyme-mediated lipid peroxidation. — Environ Health Perspect 103(Suppl 5):41–43 (1995)

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The ligninolytic fungi that cause white-rot of wood degrade a wide variety of organopollutants. The compounds metabolized include chlorophenols, chloroanilines, pesticides such as DDT and methoxychlor, and polycyclic aromatic hydrocarbons (*1,2*). To varying degrees, pollutants in these groups are co-oxidized by the fungi to give CO_2 and largely uncharacterized polar metabolites. The xenobiotic oxidations of white-rot fungi are not rapid or efficient, but they are very nonspecific. Moreover, many white-rot fungi are natural inhabitants of soil litter. These considerations make ligninolytic fungi attractive candidates for use in low tech bioremediation programs (*3*). Little is known about the mechanisms that white-rot fungi employ for organopollutant oxidation.

Xenobiotic metabolism in white-rot fungi usually parallels ligninolytic metabolism. Most recent work on ligninolysis has been done with a thermotolerant white-rotter, *Phanerochaete chrysosporium,* and we now have a fairly extensive understanding of this basidiomycete's ligninolytic mechanisms (*4,5*). Ligninolysis is oxidative, is stimulated by high oxygen levels in the culture medium, and is part of the organism's secondary metabolism; it occurs only when some nutrient, usually nitrogen, is limiting. This paucity of nutrients is the normal situation in rotting wood as well as in many soils. Lignin degradation is necessarily extracellular because lignin is a macromolecular, amorphous, highly branched, water-insoluble polymer of phenylpropane subunits. The major initial ligninolytic reaction in *P. chrysosporium* is oxidative cleavage of the polymer's propyl sidechain between C_α and C_β (Figure 1).

The initial depolymerization of lignin by *P. chrysosporium* is thought to be catalyzed by a group of related extracellular enzymes that are secreted by the fungus under ligninolytic culture conditions. These enzymes are unusually oxidizing, ferric hemoprotein peroxidases that differ significantly from commonly studied peroxidases, such as the enzyme from horseradish, in that they oxidize a much broader range of aromatic substrates. For example, these lignin peroxidases (LiPs) cleave nonphenolic lignin model dimers between C_α and C_β of their propyl sidechains to yield benzylic alcohols and aldehydes as end products, they oxidize polymethoxylated benzyl alcohols to benzaldehydes, and they oxidize various alkoxybenzenes to give benzoquinones and alkanols. A decade of research (*4,5*) has demonstrated a unified mechanism for these diverse enzymatic oxidations of aromatic compounds; they occur in one-electron steps, and postenzymatic reactions of the resulting aryl cation radicals account for the end products that are observed.

LiPs also catalyze the oxidation of certain aromatic pollutants, including various substituted phenols and polycyclic aromatic hydrocarbons (PAHs) (*2*), and could accordingly play a role in the xenobiotic metabolism of white-rot fungi. For example, certain PAHs are oxidized when they are incubated with LiP and H_2O_2 (*6,7*), with the susceptibility of a given compound to enzymatic attack depending in a straightforward way on its ionization potential (*7*). PAHs such as benz[*a*]anthracene, pyrene, and anthracene, which have ionization potentials ≤ 7.35 eV, are LiP substrates, whereas PAHs such as phenanthrene and benzo[*e*]pyrene, which have ionization potentials ≥ 7.35 eV, are not (Table 1). The products of these enzymatic PAH oxidations are PAH quinones. For example, benzo[*a*]pyrene is oxidized to its 1,6-, 3,6-, and 6,12-quinones, pyrene to its 1,6- and 1,8-quinones, and anthracene to 9,10-anthraquinone. $H_2^{18}O$-labeling

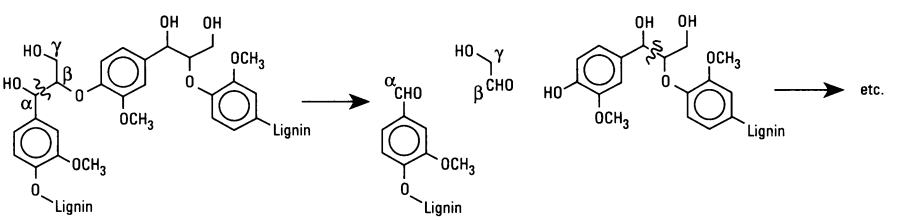

Figure 1. A linear region of lignin showing the major arylglycerol-β-aryl ether structure of the polymer and principal sites of fungal side chain cleavage.

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Table 1. Oxidation of polycyclic aromatic hydrocarbons (PAHs) by *Phanerochaete chrysosporium* lignin peroxidase.

PAH	Ionization potential (approximate, eV)	Spectral changes upon incubation with lignin peroxidase and H_2O_2
Naphthalene	8.1	-
Phenanthrene	8.1	-
Benzo[*c*]phenanthrene	7.9	-
Chrysene	7.8	-
Benzo[*e*]pyrene	7.7	-
Benz[*a*]anthracene	7.6	+
Pyrene	7.5	+
Anthracene	7.4	+
Benzo[*a*]pyrene	7.2	+
Perylene	7.1	+

Adapted from Hammel et al. (*7*).

experiments have shown, in the case of pyrene oxidation, that the new oxygens in the product quinones derive from water, presumably via nucleophilic attack of the solvent on a cationic reaction intermediate (*7*).

The PAHs present a particularly interesting research problem because their biological ring cleavage was previously considered a purely bacterial phenomenon, and ligninolytic fungi are the only eukaryotes so far known to degrade them. Anthracene will serve as an example: the initial steps of its bacterial metabolism (in pseudomonads) involve a dioxygenase-catalyzed oxidation to anthracene *cis*-1,2-dihydrodiol, further oxidation to 1,2-dihydroxyanthracene, and subsequent extradiol (*meta*) ring-fission (*8*). Previously studied fungi (e.g., *Cunninghamella elegans*), by contrast, use monooxygenases to activate the aromatic ring. The initial product in this case is anthracene-1,2-oxide, which can isomerize nonenzymatically to yield 1- or 2-anthrol or undergo enzymatic hydration to give anthracene *trans*-1,2-dihydrodiol but is not a precursor for ring cleavage (*8,9*). Neither of these pathways is likely to occur as a function of fungal ligninolytic metabolism, which ought to lead to the degradation of anthracene via 9,10-anthraquinone.

To determine whether such a new pathway exists, we investigated anthracene metabolism in *P. chrysosporium* (*10*). ^{14}C-labeled anthracene and anthraquinone (0.2 μM) were supplied to ligninolytic (nitrogen-limited) cultures, and it was shown that $^{14}CO_2$ evolution from the two compounds was similar (~13% in 14 days). The major neutral product of anthracene oxidation *in vivo* was anthraquinone, as shown by high pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC) analysis of extracted cultures and also by isotope dilution experiments. The abiotic oxidation of anthracene to the quinone was, by contrast, negligible over the time of the experiments. Both anthracene and anthraquinone were cleaved in culture to the same ring-fission product, phthalic acid, as demonstrated again by HPLC and TLC. Isotope dilution experiments confirmed this identification, showed that anthracene and anthraquinone (2.0 μM initial concentration) gave phthalate in similar yield (12–13% in 7 days), and showed that anthraquinone cleavage occurred only in ligninolytic cultures. These results show that the major pathway for anthracene degradation in *P. chrysosporium* proceeds via the quinone to phthalic acid (Figure 2) and strongly support the hypothesis that LiPs catalyze the first step of anthracene degradation in this ligninolytic fungus.

Some aromatic pollutants that are resistant to attack by LiPs are, nevertheless, degraded by *P. chrysosporium*. Certain PAHs fall within this group, phenanthrene being a notable example (*11*). Since the mechanisms responsible for these oxidations were completely unknown, we undertook the first detailed study of phenanthrene metabolism in *Phanerochaete* (*12*). The fungus oxidized [^{14}C]phenanthrene (2.0 μM initial concentration) at its 9- and 10-positions to give 10 to 15% yields of a ring-fission product, 2,2′-diphenic acid, which was identified in chromatographic and isotope dilution experiments (Figure 3). Diphenic acid formation from phenanthrene was somewhat greater in low-nitrogen (ligninolytic) cultures than in high-nitrogen (nonligninolytic) cultures, and did not occur in uninoculated cultures.

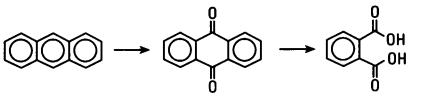

Figure 2. Known reactions of anthracene metabolism in ligninolytic *P. chrysosporium*.

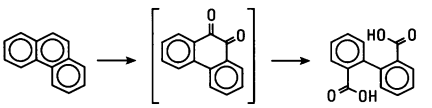

Figure 3. Oxidation of phenanthrene by ligninolytic *P. chrysosporium*. The intermediacy of the quinone is likely but not proven.

The presence of diphenic acid as a major oxidation product suggested that phenanthrene might be oxidized *in vivo* via the 9,10-quinone. Phenanthrenequinone could not be detected in cultures that were metabolizing phenanthrene but was cleaved to diphenic acid more rapidly than the PAH was in culture; therefore phenanthrenequinone is a plausible intermediate.

Subsequent work has shown that phenanthrene oxidation to diphenic acid by *P. chrysosporium* is a consequence of peroxidase-mediated lipid peroxidation. Most ligninolytic fungi secrete peroxidases whose roles are to oxidize Mn^{2+} to Mn^{3+}, which acts as a low molecular weight diffusible ligninolytic oxidant at locations remote from the enzyme active site (*13*). These manganese peroxidases (MnPs) also support Mn-dependent lipid peroxidation, and *in vitro,* MnP oxidizes phenanthrene to diphenic acid in a slow reaction that requires Mn^{2+}, oxygen, and unsaturated fatty acids (*14*). It is still not clear whether the proximal oxidant of phenanthrene is the peroxidase, a lipoxyradical, or a higher valent Mn species. Current efforts in our laboratory are therefore aimed at elucidating the reaction mechanism, which may be of general importance in the xenobiotic metabolism of white-rot fungi.

REFERENCES

1. Bumpus JA, Tien M, Wright D, Aust SD. Oxidation of persistent environmental pollutants by a white rot fungus. Science 228:1434–1436 (1985).
2. Hammel KE. Oxidation of aromatic pollutants by lignin-degrading fungi and their extracellular peroxidases. In: Metal Ions in Biological Systems, Vol 28, Degradation of Environmental Pollutants by Microorganisms and Their Metalloenzymes (Sigel H, Sigel A, eds). New York:Marcel Dekker, 1992;41–60.
3. Lamar RT, Glaser JA, Kirk TK. White rot fungi in the treatment of hazardous chemicals and wastes. In: Frontiers in Industrial Mycology, Proceedings of Industrial Mycology

Symposium, 25–29 June 1990, Madison, Wisconsin (GF Leatham, ed). New York:Routledge Chapman and Hall, 1992.
4. Kirk TK, Farrell RL. Enzymatic "combustion": the microbial degradation of lignin. Annu Rev Microbiol 41:465–505 (1987).
5. Gold MH, Wariishi H, Valli K. Extracellular peroxidases involved in lignin degradation by the white rot basidiomycete *Phanerochaete chrysosporium.* Am Chem Soc Symp Ser 389:127–140 (1989).
6. Haemmerli SD, Leisola MSA, Sanglard D, Fiechter A. Oxidation of benzo[*a*]pyrene by extracellular ligninase of *Phanerochaete chrysosporium:* veratryl alcohol and stability of ligninase. J Biol Chem 261:6900–6903 (1986).
7. Hammel KE, Kalyanaraman B, Kirk TK. Oxidation of polycyclic aromatic hydrocarbons and dibenzo[*p*]dioxins by *Phanerochaete chrysosporium* ligninase. J Biol Chem 261:16948–16952 (1986).
8. Gibson DT, Subramanian V. Microbial degradation of aromatic hydrocarbons. In: Microbial Degradation of Organic Compounds (Gibson DT, ed). New York:Marcel Dekker, 1984;181–252.
9. Cerniglia CE, Yang SK. Stereoselective metabolism of anthracene and phenanthrene by the fungus *Cunninghamella elegans.* Appl Environ Microbiol 47:119–124 (1984).
10. Hammel KE, Green B, Gai WZ. Ring fission of anthracene by a eukaryote. Proc Natl Acad Sci USA 88:10605–10608 (1991).
11. Bumpus JA. Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium.* Appl Environ Microbiol 55:154–158 (1989).
12. Hammel KE, Gai WZ, Green B, Moen MA. Oxidative degradation of phenanthrene by the ligninolytic fungus *Phanerochaete chrysosporium.* Appl Environ Microbiol 58:1832–1838 (1992).
13. Wariishi H, Valli K, Gold MH. Manganese(II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium.* Kinetic mechanism and role of chelators. J Biol Chem 267:23688–23695 (1992).
14. Moen MA, Hammel KE. Lipid peroxidation by the manganese peroxidase of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the intact fungus. Appl Environ Microbiol 60:1956–1961 (1994).